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Editorial

Progress in Evaluation of the Potential of Antisense Technology

THE CENTRAL QUESTION ABOUT ANTISENSE TECHNOLOGY has never been whether the concept is exciting, but rather whether it would work. This question has been asked in many vernaculars depending on the background of the person asking the question, but it reduces to the issue of whether oligonucleotides will have satisfactory drug properties. Will they have appropriate pharmacologic, toxicologic, and pharmacokinetic properties to realize the potential of the antisense mechanism, and is there sufficient scope for medicinal chemistry to generate new analogs with improved properties? Although definitive answers to questions about the value of drugs of any sort must await broad clinical use after marketing, the answers we have today persuasively argue that the technology should be vigorously pursued and rigorously evaluated and may work as we hope. Virtually all the data that support this view derive from studies on phosphorothioates, but exciting new chemical classes are being tested and a number are in animal studies today.

Recently, we reported on the results of Phase I/II studies of intravitreally administered ISIS 2922 in AIDS patients with refractory CMV retinitis (Crooke, 1994; Palestine *et al.*, 1994). This study demonstrated that ISIS 2922 produced a dose-dependent inhibition of progression of CMV retinitis in patients who had failed all other CMV therapy, had median CD4 counts of 4, and had a median duration of CMV retinitis of 11 months. This study also demonstrated that the drug could be given once every other week with maintenance of prolonged remissions. The main drug-related adverse event was exacerbation of ocular inflammation. Armed with this information, we are initiating definitive clinical trials for this drug. This study is exciting because it is the first study to demonstrate clinical activity of an antisense oligonucleotide and because the drug resulted in rapid, meaningful responses in desperately ill patients with virtually no other recourse. Of course, we have not yet defined the *value* of ISIS 2922 in this disease. That awaits the completion of more definitive trials. Nor does this study guarantee that ISIS 2922 will be approved as therapy for this disease. Nor can be we prove that the principal mechanism of action of ISIS 2922 in this study is antisense. Further, the study was not designed to show that antisense oligonucleotides are active when administered systemically to humans. Nevertheless, taken in the context of all other available data, these results are very encouraging.

We have also recently reported data suggesting that ISIS 2105 when injected intradermally has apparent activity in both primary and surgical adjuvant therapy of genital warts. Again, we must do much more work before we know whether ISIS 2105 is indeed active or valuable in this disease, but we are encouraged by these data as well and are initiating a multiple dose surgical adjuvant Phase II trial to confirm the activity of the drug and determine whether it has sufficient value to be commercialized.

We have reported definitive pharmacokinetic studies on ISIS 2105 in rats after intravenous and intradermal doses (Cossum *et al.*, 1993, 1994). These studies clearly demonstrate excellent bioavailability, peripheral tissue distribution, and clearance that support once a day or every other day dosing. Similar results have been reported by the group at Dupont Merck (Sands *et al.*, 1994), and they have shown autoradiographic results showing drug inside cells in the liver and kidney. We and our colleagues at Ciba-Geigy have similar autoradiographic data, not yet published. Furthermore, we will shortly report definitive pharmacokinetic studies after intradermal dosing in man confirming that man and rats (as well as monkeys, mice, and rabbits) handle phosphorothioates similarly (Crooke *et al.*, 1994). These data are extremely important as they demonstrate attractive parenteral pharmacokinetic properties for phosphorothioate antisense drugs and show that for this class of drugs *in vitro* cell uptake studies do not predict *in vivo* behavior. This last point is not surprising as there is no class of drugs of which I am aware whose pharmacokinetic properties can be simply extrapolated from *in vitro* studies.

In a wide range of studies performed in our laboratories, Hybridon's and others, we have also defined the toxic liabilities of phosphorothioates. We believe the dose limiting toxicities will likely be related to effects on clotting, complement activation, or possibly cytokine release, and that the therapeutic index will be satisfactory. We will also shortly report studies that define the mechanisms underlying these effects.

Perhaps most importantly, however, we and many other laboratories have demonstrated potent systemic effects of phosphorothioates in animals in which all of the data are consistent with an antisense mechanism (Hijiya *et al.*, 1994; Skorski *et al.*, 1994). In our laboratories and those of our collaborators, we have shown potent antisense activities against Ha-RAS, Ki-RAS, PKC-α, RAF kinase, ICAM-1 and other targets. In several cases, we have unequivocally proven mechanism by showing a selective loss of target RNA in various tissues at doses ranging from less than 1 mg/kg to 20 mg/kg daily. Interestingly, because the cells that expressed many of the targets listed above did not take up sufficient oligonucleotide, we had to employ cationic lipid transfection to show *in vitro* activity. *In vivo, no*

specialized delivery system was required. In a particularly important series of studies, Dean *et al.* (1994) have shown potent systemic isotype selective loss of PKC-α RNA induced by a phosphorothioate oligonucleotide and shown 24-hour duration of effects and absence of tachyphylaxis.

In aggregate, all of the data encourage cautious optimism.

So does this mean that the "bullets are really magic"? In my view, this question epitomizes one of the causes of cynicism regarding antisense technology. We are developing a new pharmacological and chemical class of drugs. That we are simultaneously creating a new technology and trying to develop drugs from this technology is entirely appropriate and the only real way to understand the drug properties of these molecules. We hope these drugs will be of unique value. They are, nevertheless, drugs. We expect them to have a variety of effects, but if we can understand these properties in the context of modern pharmacology and antisense drugs continue to perform as well as they have to date, then patients will benefit. With these drugs, as with all other classes of drugs, there will be questions that cannot be answered. However, we have already generated more *direct* proof of mechanism of action in animals than for many more established classes of drugs, and the pharmacokinetic and toxicologic properties appear, at present, to be reasonably attractive.

That there are questions that we cannot answer definitively should not be cause for despair. Do we know the precise mechanisms that explain aspirin uptake into cells? Do we understand, at a biochemical level, how aspirin disproportionates between serum protein binding sites and peripheral tissues? For how many classes of drugs do we have unequivocal direct proof of mechanism of action in animals or man?

I would urge continuing critical evaluation of antisense technology. We need to continue to try to understand these drugs. We expect that we will find limits to their utility. For example, we already know that phosphorothioates do not cross an intact blood brain barrier and are minimally orally bioavailable. We may even unearth effects that negate the potential of this technology entirely. However, the data to date are encouraging, and the technology has successfully overcome a large number of hurdles in a relatively short time.

On the other hand, I would hope for an end to the cynicism about antisense technology. This begins with asking the right questions in the right way. The right questions pertain to factors influencing therapeutic index and the breadth and ease of therapeutic use. The right way to ask the questions is in the context of modern pharmacology and in carefully controlled experiments in which dose response curves for various effects are critically defined.

In short, we must set reasonable expectations for this technology, evaluate its potential reasonably and report our results with integrity. If we do this, we will meet our responsibilities to the technology, to patients in need, and, for those of us in commercial organizations, to investors.

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